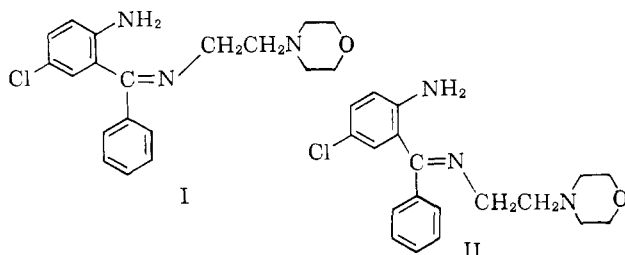


benzophenone and aminoethylmorpholine in xylene under reflux in the presence of zinc chloride. Following solvent removal, fractional crystallization of the residue from alcohol and hexane gave a less soluble α -form I, m.p. 140–142° (*Anal.* Calcd. for $C_{19}H_{22}ClN_3O$: C, 66.36; H, 6.45; N, 12.22. Found: C, 66.13; H, 6.29; N, 12.09.) and a more soluble β -form II, m.p. 112–114° (Found: C, 66.17; H, 6.50; N, 12.43). Both I and II upon treatment with aqueous acid returned the starting materials. Heating II without a solvent at 140–150° for 10 min. afforded I.



The infrared and n.m.r. spectra of I and II were consistent with the imine formulations.

Compounds I and II had different ultraviolet absorption spectra: I, λ_{\max}^{EtOH} 248 $m\mu$ (ϵ 25,300); II, λ_{\max}^{EtOH} 233 (ϵ 27,100), 362 $m\mu$ (ϵ 4,840). The α - and β -oximes of 2-amino-5-chlorobenzophenone (III and IV) whose configurations have been firmly established³ as *syn* and *anti*, respectively, to the substituted phenyl had the following ultraviolet absorption spectra: III, λ_{\max}^{EtOH} 246 $m\mu$ (ϵ 21,700); IV, λ_{\max}^{EtOH} 231 (26,000); 346 $m\mu$ (ϵ 8,500). Compound I, therefore, has a *syn* configuration to the substituted phenyl with II having an *anti* arrangement. The absorption bands above 320 $m\mu$ in II and IV are believed to be due to the chromophores arising from conjugation of the *o*-aminophenyl group with the imino bond which is possible only when the nitrogen substituent is *anti*. Steric crowding does not permit the rings to be coplanar.⁴

The assigned configurations are supported by the absorption spectrum of N-(2-amino-5-chloro- α -phenylbenzylidene) glycine, sodium salt, $\lambda_{\max}^{H_2O}$ 240 $m\mu$ (ϵ 17,500), which was obtained⁵ by opening 7-chloro-5-phenyl-1,3-dihydro-2H-1,4-benzodiazepin-2-one with alkali and which must have its nitrogen substituent *syn* to the amino substituted phenyl group.

Further, the absorption spectrum of benzophenone morpholinoethylimine, b.p. 180–183° (0.3 mm.) (*Anal.* Calcd. for $C_{19}H_{22}N_2O$: C, 77.51; H, 7.53; N, 9.52. Found: C, 77.74; H, 7.54; N, 9.23.), in which the imino bond can only be in conjugation with an unsubstituted phenyl group, exhibited no maximum above 300 $m\mu$. This is true also for benzophenone oxime.

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(4) P. A. S. Smith and E. P. Antoniadis, *Tetrahedron*, **9**, 210 (1960).

(5) S. C. Bell, T. S. Sulkowski, C. Gochman, and S. J. Childress, *J. Org. Chem.*, **25**, 562 (1962).

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Comparison of Hypochromic Effect and Optical Rotation in Poly-L-glutamic Acid

Sir:

We wish to report some data which resolve a controversy over the relationship between the helical conformation of polypeptides and the hypochromic effect in the amide absorption band at 190 $m\mu$ discovered by

Imahori and Tanaka.¹ The idea that the decrease in the extinction coefficient at this wave length could be simply correlated with the extent of helix formation was called into question by Wada,² Applequist,³ and Miyazawa⁴ on the grounds that the extinction coefficient for poly-L-glutamic acid (PGA) was not simply correlated with the optical rotation and in fact seemed to follow more nearly the degree of ionization of the carboxyl groups. It was recognized, however, that the various experiments being compared were carried out under different conditions, particularly regarding concentration of polypeptide, and that the results of the comparison were therefore inconclusive. In order to obtain more decisive information, we have carried out optical rotation, ultraviolet absorption, and titration measurements on identical solutions of PGA.

PGA (Pilot Chemicals Lot G-13, intrinsic viscosity = 1.56 dl./g. in 0.2 *M* NaCl, pH 7) was dissolved with the aid of dilute NaOH to a concentration of 0.50 g./dl. (based on carboxyl form) in the presence of 0.2 *M* NaCl. Titration of the solution with 0.8 *M* HCl was followed with a Beckman Model G pH meter. The volume change due to addition of acid from pH 7 to 4 was less than 4% and was not corrected for in the concentration. Optical rotations at 589 $m\mu$ were measured with a Rudolph spectropolarimeter in a 20-cm. polarimeter tube, giving measured rotations from -0.1° to -1° with a precision of $\pm 0.01^\circ$. Absorption spectra were measured on a Cary Model 14 spectrophotometer in a cell of 0.01 cm. path length (Quaracell Products, Inc.). At 190 $m\mu$ absorbances were in the range of 2 to 3. Large errors at this wave length due to noise and chloride absorption (arising from slight mismatching of sample and solvent cells) precluded accurate work, and we chose instead to make measurements on the side of the absorption band, where the hypochromic effect is still easily observable. The wave length 200 $m\mu$ seemed optimum for this purpose, as the absorbances were in the range of 1 to 2 and the precision ± 0.005 . Extinction coefficients showed less precision, however, due to variations in path length which occur when the cell is dismantled for refilling. Rotation and extinction coefficient measurements were therefore of comparable precision.

The results are shown in Fig. 1. It is seen that the change in extinction coefficient follows rather closely the change in rotation, and that neither of these is a linear function of the degree of ionization. It, therefore, appears that the discrepancies noted earlier can be attributed to differences in conditions of measurement. Furthermore, it is now evident that Imahori and Tanaka were correct in attributing the hypochromic effect to interactions in the helical conformation, since the change in rotation has been shown⁵ to result from helix formation at low pH. This conclusion is of particular interest in view of the success of Tinoco, *et al.*,⁶ in predicting the magnitude of the hypochromic effect from dipole-dipole interactions in the helix. It does not follow from these observations that either rotation or absorption is linearly related to the helix content, but merely that both are similar functions of helix content. The linear relationship generally assumed is neither confirmed nor contradicted.

(1) K. Imahori and J. Tanaka, *J. Mol. Biol.*, **1**, 359 (1959).

(2) A. Wada, in "Polyamino Acids, Polypeptides, and Proteins," M. A. Stahmann, Ed., University of Wisconsin Press, Madison, Wisconsin, 1962, p. 157.

(3) J. Applequist, *ibid.*, p. 158.

(4) T. Miyazawa, *ibid.*, p. 159.

(5) P. Doty, A. Wada, J. T. Yang, and E. R. Blout, *J. Polymer Sci.*, **23**, 851 (1957).

(6) I. Tinoco, Jr., A. Halpern, and W. T. Simpson, "Polyamino Acids, Polypeptides, and Proteins," M. A. Stahmann, Ed., University of Wisconsin Press, Madison, Wisconsin, 1962.

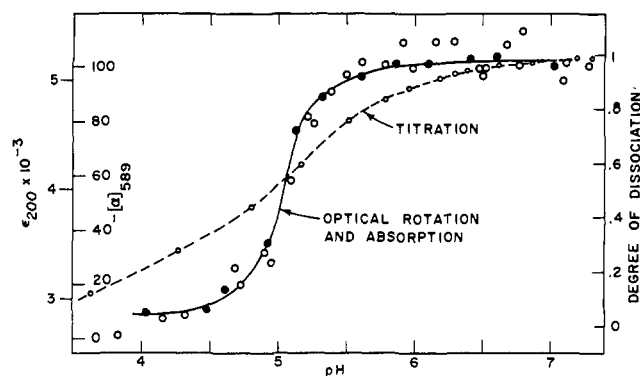


Fig. 1.—Optical rotation, ultraviolet absorption, and titration of poly-L-glutamic acid, 0.5 g./dl. in 0.2 *M* NaCl. (O), Molar (residue) extinction coefficient at 200 *mμ*, ϵ_{200} . (●), Specific rotation at 589 *mμ*, $[\alpha]_{589}$. Degree of dissociation of carboxyl groups is shown by titration curve. Ordinate scales are adjusted to make the ranges of the various measurements approximately coincide.

In the absorption measurements reported here no attempt has been made to separate the changes in carboxyl absorption with pH from the changes in amide absorption. A rough experiment on acetic acid under conditions similar to those used for PGA showed $\epsilon_{200} = 260$ at pH 9 and $\epsilon_{200} = 130$ at pH 4. These values are relatively small, and an attempt to correct for carboxyl absorption would not be expected to affect our conclusions.

It will be noted that our curve showing absorption vs. pH is markedly different from that observed by Imahori and Tanaka under the conditions of their measurements (unspecified). They found ϵ_{190} to change gradually from pH 4 to 8, with an intermediate plateau from about pH 4.5 to 6. No such plateau is evident from our data, and the transition is sharper. It is unlikely that the difference is due to the choice of wave length, as we find similar transition curves at all wave lengths in the portion of the band accessible to accurate measurements. It will also be noted that the optical rotation reaches a constant value at the low pH side in our data, whereas other experimenters^{2,7} have observed a maximum in the rotation at about pH 4.5. This difference may be due to unknown factors in the particular samples or handling techniques. Solutions at about pH 4 are, for example, known to be metastable, and precipitation sometimes produces irreproducible results. No precipitation was evident above pH 4 in our experiments.

Acknowledgment.—This investigation was supported by a Public Health Service research grant (GM-10882-03) from the Division of General Medical Sciences, Public Health Service.

(7) M. Idelson and E. R. Blout, *J. Am. Chem. Soc.*, **80**, 4631 (1958).

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Vicinal Proton Coupling in Nuclear Magnetic Resonance¹

Sir:

Considerable attention has focused recently on the utilization of the dihedral-angle dependence of vicinal proton coupling constants for structural studies of

(1) Most of the calculations reported in this paper were made on ILLIAC at the University of Illinois during the year 1959–1960 and reported at the American Chemical Society Symposium on High Resolution n.m.r. held at Boulder, Colorado, July 2–4, 1962.

organic molecules.² Since most of the investigations have been experimental in character, it is helpful to recall the theoretical foundations of the subject. This seems to be of particular relevance at the present time because of published statements³ (e.g., the implication that the vicinal coupling constants are expected theoretically to depend *only* on the dihedral angle) that indicate some confusion concerning the predictions and the limitations of the theory. In this communication we outline the theoretical results for vicinal proton couplings in ethanic, ethylenic, and related systems. Special emphasis is placed on the factors that are expected to alter the behavior from that determined for the unperturbed model used in the original calculations.⁴

The valence-bond σ -electron calculation,^{4,5} which utilizes a nonionic six-electron, six-orbital fragment (HCC'H') to determine the contact interaction, yields results that can be approximated by the equation⁶

$$J_{HH'} = A + B \cos \phi + C \cos 2\phi \quad (1)$$

where ϕ is the dihedral angle. For a C–C bond length of 1.543 Å., sp^3 hybridized carbon atoms, and an average energy (ΔE) equal to 9 e.v., the constants are $A = 4.22$, $B = -0.5$, and $C = 4.5$ c.p.s. A fragment with sp^2 hybridization, a C–C bond length of 1.353 Å., and the same ΔE value gives $J_{HH'}(cis) = 6.1$ c.p.s. and $J_{HH'}(trans) = 11.9$ c.p.s. These results appear to be in qualitative agreement with some of the salient features of the measured couplings (e.g., the dihedral-angle dependence in ethanic compounds, the *cis/trans* ratio in ethylenic compounds.^{7,8} However, the numerical values obtained from the calculations are to be regarded as only "zero-order" approximations. As pointed out originally,⁹ "the variability of the observed coupling constants is not predicted by the simple model considered in this paper. Refinements, such as the inclusion of electron orbital and dipolar electron spin terms, a more careful choice of integral values, and the consideration of ionic and other perturbations, should be introduced. Also, a determination of the θ dependence [HCC' angle] and the C–C bond-length dependence of the coupling constant, which are only hinted at here by the differences between ethane and ethylene, would be of value for the interpretation of various ring-system spectra." In what follows we provide estimates of some of these effects. As in our previous work, this is done primarily to suggest the trends that are expected on theoretical grounds.

Ionic Substitution Perturbations.—If a substituent is introduced whose electronegativity (X) is different from hydrogen (X_H), significant deviations from the

(2) See, for example, C. D. Jardetsky, *J. Am. Chem. Soc.*, **83**, 2919 (1961); R. U. Lemieux, *Can. J. Chem.*, **39**, 116 (1961); R. W. Lenz and J. P. Heesch, *J. Polymer Sci.*, **51**, 247 (1961); R. J. Abraham and K. L. McLauchlan, *Mol. Phys.*, **5**, 513 (1962); N. J. Leonard and R. A. Laursen, *J. Am. Chem. Soc.*, **85**, 2027 (1963).

(3) See, for example, (a) K. L. Williamson, *ibid.*, **85**, 516 (1963); (b) O. L. Chapman, *ibid.*, **85**, 2014 (1963); G. V. Smith and H. Kriloff, *ibid.*, **85**, 2016 (1963); P. Laszlo and P. von R. Schleyer, *ibid.*, **85**, 2018 (1963).

(4) M. Karplus, *J. Chem. Phys.*, **30**, 11 (1959).

(5) H. Conroy, "Advances in Organic Chemistry," Vol. 11, Interscience Publishers, Inc., New York, N. Y., 1960, p. 265, gives a molecular orbital treatment.

(6) Equation 1 is similar to, but slightly more accurate than, the original equation (eq. 11, ref. 4). Corresponding expressions have recently been suggested by J. Powles, *Discussions Faraday Soc.*, **34**, 30 (1962); M. Barfield and D. M. Grant (private communication); and O. Jardetsky (private communication).

(7) For a recent review, see C. N. Banwell and N. Sheppard, *Discussions Faraday Soc.*, **34**, 115 (1962).

(8) The theoretical positive sign for most vicinal couplings is probably confirmed by the measurement of K. L. McLauchlan and A. D. Buckingham (private communication) of $J_{HH'}(ortho)$ in *p*-nitrotoluene, though additional relative sign determinations are needed [e.g., $J_{HH'}(ethyl)$ vs. $J_{HH'}(ortho)$].

(9) Ref. 4, p. 15.